**Department of Energy**
Bonneville Power Administration
Public Involvement
P.O. Box 12999
Portland, Oregon 97212

In reply refer to: PJS

TO: Interested Parties

SUBJECT: Fish Health and Culture Program Area Review--1984

Bonneville Power Administration (BPA) held a public meeting on March 7 and 8, 1984, to review 18 research and development projects funded by BPA concerning fish health and fish culture. One of the purposes of this meeting was to familiarize the various entities with these projects. Other purposes included information exchange, constructive comments, and coordination with other projects. The results proved both stimulating and productive, with the result that we expect to repeat this next year.

The following pages list each Project Title, Project Number, Project Leader and their location, BPA's Project Officer (Contracting Officer's Technical Representative), and a brief summary of the project. Remember: these summaries are preliminary results; they are subject to change, and should not be quoted without consulting the Project Leader.

As promised at the meeting, this information was assembled and is being disseminated to further facilitate the above goals. If you have any questions, please feel free to contact the respective Project Leader or call me at (503) 230-5199.

Sincerely,

G.R. Bouck

Gerald R. Bouck, Ph.D
Program Area Manager for
Fish Health and Fish Culture

Enclosure

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ANNUAL REVIEW OF BPA-FUNDED PROJECTS
IN
FISH HEALTH AND FISH CULTURE

March 7 and 8, 1984, 9:00 a.m.

Cosmopolitan Airtel
6221 NE. 82nd Avenue, Apollo Room
Portland, Oregon 97220

GBouck:ay (WP-PJS-3129N)

PROJECT SUMMARIES

## 1.0 FISH CULTURE SECTION

### 1.1 ESTIMATION OF ARTIFICIAL PROPAGATION POTENTIAL IN THE COLUMBIA RIVER BASIN (PROJECT NO. 83-424); (Project Officer: Jerry Bouck)

Phase I was completed before the Project manager and staff resigned to begin employment elsewhere. A list of anadromous fish hatcheries was compiled, agency contacts were established, standarized data forms were developed, and most methods were resolved. A new contractor will be selected to conduct the data collection in Phase II.

### 1.2 LOW-COST SALMON AND STEELHEAD PRODUCTION SYSTEM FOR THE COLUMBIA RIVER BASIN (PROJECT NO. 83-353); Harry Seen, Fish Management Consultants 5211 Blvd. Ext. Road, Olympia, Washington 98501; (206) 352-3033, (206) 491-0579; (Project Officer: Jerry Bouck)

The objectives of this study are:

1. To produce a compendium of low-capital propagation facilities;

2. Identify costs, vendors, sources of information, and locations where the units can be seen;

3. Identify cost-saving operational techniques and tools; and

4. Illustrate how each low capital propagation facility can or cannot be incorporated into the goals of the Columbia River Basin.

The project consists of three phases:

1. Planning;

2. data gathering and compilation; and

3. report preparation.

In Phase I, planning, Fish Management Consultants prepared a study plan outline which detailed the proposed contents of the report. This was circulated to all the Columbia Basin Fisheries agencies and Tribal communities for their review and input. Prior to this, FMC had developed lines of communication with their intended parties and requested that a contact person be named who would be the liaison with FMC on the project. FMC then met with the contact people and discussed the intent of the final report and obtained input. A suggested report outline incorporating agency and tribal views was then submitted to BPA and after their review, FMC developed and agreed upon the final report contents and format.

Phase II, data compilations, consisted of a literature review and field visits to fish agencies and tribal hatcheries. Over 80 stations in Washington, Oregon, Idaho, and British Columbia were visted to obtain information. Contacts were also made in Alaska, and information gathered from programs in Japan and Norway will be included in the final report. Data gathering was essentially completed in November.

Phase III, report preparation, is well underway. Drafts of each chapter have been completed, and we are currently re-drafting and editing as necessary. The final report is due May 30, 1984, and we fully expect to meet this agreed upon date.

1.3 LOW-TECHNOLOGY FISHERIES FACILITIES (PROJECT NO. 83-350); James H. Johnson, Nez Perce Tribe, Lapwai, Idaho 83540; (208) 935-2253; (Project Officer: Tom Vogel)

Measure 704 (j)(2) in the Columbia River Basin Fish and Wildlife Program refers to the construction, operation, and maintenance of low-capital propagation facilities on the Nez Perce Reservation. Consequently, BPA provided funds to the Nez Perce Tribe to initiate Phase I of this study. Phase I will include (a) identification, evaluation, and ranking of potential sites for low technology artificial propagation, rearing, acclimation, and adult capture and juvenile release facilities for spring and fall chinook salmon, coho salmon, and steelhead; (b) development of an integrated low technology artificial propagation conceptual plan based upon the selected sites and anadromous fish production goals; and (c) preliminary design, cost estimates, and estimated construction schedule for the recommended fish facilities. It is anticipated that Phase I, which got underway in January 1984, will be completed by September 1984, Phase II is earmarked to include final design and construction while Phase III will be operation and maintenance.

As of the end of February 1984, progress on Phase I has included issuance of RFP's, selection of CH2M Hill Engineers (Boise, Idaho) as the principal subcontractor, and finalization of the subcontracting agreement. In mid-February a tour of potential sites on the Reservation was provided to CH2M Hill staff by Nez Perce Tribal fisheries staff. Further on-the-ground investigations are planned in mid-March. Presently, efforts are being made to develop criteria for site prioritization.

1.4 DEVELOPMENT OF DIETS FOR ENHANCED SURVIVAL OF SALMON (PROJECT NO. 83-363); David L. Crawford, Oregon State University, Seafoods Laboratory; 250-36th Street, Astoria, Oregon 97103; (503) 325-4531; (Project Officer: Tom Clune)

SUMMARY

The influence of feed regimes is being determined on the efficiency of hatchery production, and on the survival or return of coho and chinook salmon to the Columbia River system. Our hypothesis is that high quality fish protein in feeds, yield higher quality smolts with superior survival characteristics.

A supply of vacuum-dried fish and bone-free spray dried fish hydrolysates was developed through direct cooperation with a commercial firm. Facilities and capabilities for producing rations was expanded through equipment acquisition and/or fabrication and construction of a 24' x 50' building equipped with 12' x 22' sharp freezer.

Balanced diet formulations were developed which provide a minimum of 55 percent protein (dry weight) and sufficient fat energy for a near 1:1 caloric ratio with total protein. A flexible formulation was eventually adopted for vacuum-dried salmon to accommodate its marked variation in fat content.

Laboratory-scale feeding trials were planned and carried out to define the growth response of young salmon to high quality fish protein in relation to commercial herring meals dried under rigorous temperature conditions. Results for large fall chinook fingerlings revealed no marked difference between vacuum-dried meals and high quality commercial herring meal. Vacuum-dried salmon meal produced a slightly better growth response than a similarly prepared meal of hake. Laboratory starter ration (mash through 1/32" pellet size) investigations were initiated in late December 1983. Growth response are being compared between vacuum-dried meals of round salmon and hake and steam tube dried salmon. The intact protein content of these meals is also being compared to their hydrolyzed and spray-dried counterparts, as well as compared to products prepared from ground fish carcass waste. The following hydrolyzed "wet fish" components are being tested in direct comparison to water for their potential in improving feed acceptance by young fry: krill, beef liver, tuna viscera, shrimp, and squid. Growth produced by vacuum-dried meals and spray-dried hydrolysates will be studied with small fall chinook fingerlings in mid-February 1984. Preliminary investigations indicate that vacuum-dried salmon meal yields a remarkably rapid growth rate for this size of fish.

Hatchery-scale survival investigations were initiated with coho salmon at Oregon Department of Fish and Wildlife's Sandy River hatchery and with fall chinook salmon at the Bonneville Dam hatchery. Coho survival investigations were initiated on June 27, 1983, utilizing duplicate ponds of 59,000 fish. Test diets contained vacuum-dried salmon and hake meal, and are being compared to the Oregon moist pellet regime. Between October 27 and November 4, 1983, 27,000 fish/pond were injected with coded-wire tags and marked with an adipose fin clip. Fish are being fed at a rate which will produce a size approximately 25 g/fish at a May 1, 1984, release time. Duplicate groups of 600,000 fall chinook will be demand-fed either a test ration containing vacuum-dried salmon meal or Oregon Moist Diet (control). When the fish weigh 250-300 fish/lb, they will be coded-wire tagged and marked with an adipose fin clip. A May 1, 1984, release date is projected.

1.5 STOCK IDENTIFICATION OF COLUMBIA RIVER CHINOOK SALMON AND STEELHEAD TROUT (PROJECT NO. 83-451); Carl B. Schreck, Oregon Cooperative Fishery Research Unit, 104 Nash Hall, Oregon State University, Corvallis, Oregon 97331; (503) 754-4531; (Project Officer: Jerry Bouck)

This study is identifying unique stocks of Columbia River steelhead trout and chinook salmon in Oregon and Washington. Each wild and hatchery stock of chinook salmon and steelhead trout will be characterized using gene frequencies based on electrophoretic characters, life history characters (such as time of return and disease resistance), and morphological characters (such as taxonomic measurements and counts). The degree of similarity between the stocks will be ascertained by cluster analysis. Stock characteristics will be correlated with environmental variables of the home streams.

The benefits from this study will be: (a) identification of stocks requiring unique management decisions; (b) protection of the genetic integrity of the existing stocks; and (c) a basis of selecting donor stocks to restore depleted runs.

To date 19 steelhead trout stocks and 16 chinook salmon stocks have been sampled, and 17 steelhead stocks have been analyzed by electrophoresis.

1.6 PROTECTION OF WILD ADULT STEELHEAD IN THE UPPER SNAKE RIVER OF IDAHO AND EVALUATION OF ITS EFFECTIVENESS (PROJECT NO. 84-02); David W. Ortman, Idaho Department of Fish and Game, 600 South Walnut, P.O. Box 25, Boise, Idaho 83707; (Project Officer: Jerry Bouck)

The large quantity of hatchery-reared steelhead trout that are mingled with wild stocks presents a need for easy identification of wild vs. hatchery fish in the fishery. Identification of hatchery fish by removal of the adipose fin is being used in this project to facilitate protection of wild stocks while permitting a liberal harvest of the more abundant hatchery stocks.

After clearing the fin mark through the Columbia Basin fishery agencies, all steelhead in Idaho scheduled for release in the spring of 1984 were given the fin clip in the winter of 1983-84. Nearly 5 million had been marked at the time of this reporting, and the year's total will exceed 5 million at the job's end. Overall mortality in the hatcheries during the work was less than 1 percent.

This technique will continue to be used through the foreseeable future. Evaluation will be conducted by monitoring the fishery and gauging the value of the fin mark to selective management of stocks in the mixed-stock fisheries.

SUMMARY OF STEELHEAD ADIPOSE FIN CLIPPING

1984 SMOLT RELEASE

| HATCHERY | NO. AD CLIPPED | DAYS | MAN DAYS 1/ | NO. FISH/ MAN DAY | MORTALITY | NO./LB |
|---|---|---|---|---|---|---|
| DNFH | 1,706,800 | 8 | 448 | 3,810 | 10,000 | 15.4-48.5 |
| HGNFH | 1,312,600 | 16 | 423 | 3,103 | 26,000 | 2.6-7.5 |
| MAGIC VALLEY | 234,600 | 12 | 52 | 4,512 | 600 | 24.7-31.0 |
| NIGARA SPRINGS | 1,689,200 | 24 | 390 | 4,331 | 5,000 | 6.0-20.0 |
| TOTAL | 4,943,200 | | 1,313 | 3,765 | 41,600 | |

1/ Includes set-up, take-down, hatchery help, supervisory time.

1.7 PEN REARING AND IMPRINTING OF FALL CHINOOK SALMON (PROJECT NO. 83-313); Jerry J. Novotny, Fishery Biologist, U.S. Fish and Wildlife Service, Seattle National Fishery Research Center, Willard Field Station, Cook, Washington 98605; (509) 538-2299; (BPA Project Officer: Tom Clune)

The feasibility of imprinting adult fall chinook to sites in the upper Columbia basin will be evaluated by rearing age 0 fish, monitoring their growth and survival, and releasing them from enclosures located in a backwater and an acclimation pond in the John Day pool. Optimum feeding rates and stocking densities will be determined and cost/benefit of offstation rearing will be compared to hatchery production. Results of the study will be applicable to offstation rearing throughout the Columbia basin.

Backwaters and protected sites located along the Columbia River between John Day and Priest Rapids dams, and the lower reaches of the Umatilla, Yakima, and Snake rivers were surveyed to determine their suitablility for experimental rearing of age 0 fall (upriver bright) chinook salmon. Twenty-six potential study sites were judged as unusable based on criteria which included depth, area, accessibility, potential water level and temperature fluctuations, entrance-access to the river, public use, and obvious water quality problems. Eight remaining sites were then thoroughly evaluated to determine suitability for rearing studies, using water quality and biological data to supplement physical observations. The criteria used in the final selection of rearing sites included an assessment of water source, depth, temperature, and quality, proximity to natural spawning sites, ease of adult capture, and benthos and zooplankton abundance.

Two sites were selected as satisfying the most criteria for experimental rearing studies: Rock Creek (river km 337) and Social Security Pond (river km 468). All other sites surveyed were ranked as either less desirable, or unusable for these studies.

1.8 SNAKE RIVER FALL CHINOOK SALMON BROOD PROGRAM (PROJECT NO. 82-7); Lee W. Harrell, NMFS - Box 38, Manchester, Washington 98353; (206) 842-7181; (BPA Project Officer: Richard Harper)

The Snake River Fall chinook salmon broodstock program is based on captive broodstock whose progeny are reared to maturity and their resultant eggs used as an adjunct to restoring this depleted run.

Inventory - February 24, 1984

| | | | | |
|---|---|---|---|---|
| 1980 | brood chinook salmon | 1,208 | 1150.0 gm | Manchester |
| 1981 | brood chinook salmon | 3,753 | 300.0 gm | Manchester |
| 1982 | brood chinook salmon | 5,980 | 17.3 gm | Manchester |
| 1983 | brood chinook salmon | 15,500 | fry | Big Beef Creek |

Most of these fish were smolted and transferred to marine net-pens as 1+ age fish. Using a newly constructed freshwater lens system, fish were acclimated fish to full strength seawater during January or at approximately 1 year of age. After the brood fish are acclimated and growing in marine net-pens, very few losses were attributed to known pathogens. However, after 15 to 18 months in seawater residence, we discovered a previously undocumented marine disease that is a serious threat to the chinook salmon broodstock programs. For example, since September 1983, this tentatively identified fungal disease has resulted in the loss of several thousand 1980 brood fish in marine net-pens. Concurrent to the production program, we conduct research on broodstock nutrition, disease, smoltification (acclimation), and spawning strategies. A small number of the 1980 brood is expected to mature this fall (1984) and will be used to determine optimum spawning procedures for subsequent years, provided that new marine diseases cannot be transferred to offspring via infected gametes.

## 2.0 FISH DISEASE SECTION

### 2.1 RAPID DIAGNOSIS OF IHN VIRUS INFECTION IN SALMON AND STEELHEAD TROUT (PROJECT NO. 82-20); Jo-Ann C. Leong, Oregon State University, Corvallis, Oregon 97331; (503) 754-4441; (Project Officer: Jerry Bouck)

A diagnostic test for infectious hematopoietic necrosis virus (IHNV) has been developed. The test involves the radiolabeling of virus-specific proteins with 35S-methionine. It yields two pieces of information at once: (a) a confirmation of IHNV infection without the necessity of a neutralization test; and (b) identification of the IHNV strain. This finding that IHNV strains may be typed by the virion protein patterns has allowed us to make the following findings:

1. A particular strain of IHNV is characteristic for a specific geographic area and not for a particular species of fish.

2. The same strain of virus is found in the tissues of spawning adult fish and in the progeny of these fish during an IHNV epizootic.

3. There are at least five different groups of strains of IHNV. These include:

Strain 1: Found in kokanee and sockeye salmon in Alaska, British Columbia, and the Columbia River basin. Found in steelhead and chinook salmon in the Deschutes River system. It is characterized by a fast migrating N protein.

Strain 2: Found in steelhead trout, cutthroat trout, and chinook salmon in the Columbia River basin and in rainbow trout in Idaho. It is characterized by a N protein which migrates at approximately 41,000 daltons.

Strain 3: Found in chinook salmon is California coastal hatcheries and in rainbow trout at Nan Scott Lake in Oregon. It is characterized by a N protein that migrates at approximately 43,000 daltons.

Strain 4: Found in chinook salmon at Coleman Hatchery on the Sacramento River system. It is the only strain with a slower migrating G protein and it is temperature sensitive.

Strain 5: Strains that do not fit into any specific grouping at this time.

4. Outbreaks of IHNV among fish in the Lower Columbia River were caused by the same strain. This strain is identical in its protein pattern to strains isolated from rainbow trout in Idaho.

5. A similar test for diagnosing IHNV infection has been developed with immunological reagents.

SIGNIFICANCE: The finding that IHNV strains may be typed by the virion protein patterns and that a particular strain is enzootic for an area is significant for several reasons. The most important reason is that once an area has been "typed" with a particular strain, it becomes possible to detect the introduction of a new strain into the area. If the appearance of a new strain of IHNV into an area coincides with the introduction of fish and eggs, then it may be possible to trace the origin of the virus. It should be possible to distinguish between an IHNV epizootic which originated from contaminated eggs or from infected fish in the watershed above the hatchery.

2.2 DEVELOPMENT OF RAPID SERODIAGNOSTIC TESTS FOR THE DETECTION, SURVEILLANCE, AND DIAGNOSIS OF FIVE IMPORTANT PATHOGENS OF FISHES IN THE COLUMBIA RIVER BASIN (PROJECT NO. 83-304); Dan Mulcahy and Pete Bullock, U.S. Fish and Wildlife Service, National Fishery Research Center, Bldg. 204, Naval Station, Seattle, Washington 98115; (206) 527-6282 or (FTS) 446-6282; (Project Officer: Jay Dugoni)

The enzyme-linked immunosorbent assay (ELISA) is a rapid and sensitive immunologically based antigen detection method. The goal of this project is the development of ELISA tests for the bacteria causing bacterial kidney disease (BKD), enteric redmouth (ERM), furunculosis, and the viruses causing infectious hematopoietic necrosis (IHN) and infectious pancreatic necrosis (IPN). Work in the first year concentrated on antigenic analysis and antisera development.

Laboratory evaluation of the ELISA test for the BKD pathogen (Renibacterium aslmoninarum) is near completion, and field testing will begin in the Summer 1984. The test detects an antigen shown to be present in liver, kidney, feces, and serum of infected fish. Current detection limits are in the 2-20 ng range, with a processing time of approximately 6 hours.

Ion exchange chromatography of cultures of both types of the agent causing ERM (Yersinia ruckerii) has indicated that there are five proteins present in each. One protein is a cross-reacting antigen common to both types. Rabbits have been immunized with Type I extracellular products and with Type II cells. Antigent analysis and antiserum development will continue in 1984.

Presently available antisera to Aeromonas salmonicida, the causative agent of furunculosis, cross-reacted with A. hydrophila isolates. Antigenic analysis of extracellular antigens showed a protein with a molecular weight of 180,000 which did not cross-react. This antigen has been purified and an antiserum produced. Preliminary ELISA test development with the antigen and antibody give a detection limit of 10 ng.

A good quality IPN virus antiserum was used in preliminary ELISA development and gave a detection limit of $10^5$ pfu per ml, suitable for diagnosis but not for carrier detection. Antisera against viral proteins will be developed to increase sensitivity and reduce background levels. Because of the poor quality of antisera to IHN virus available, hybridoma cells are being developed for ELISA use.

The second year's goal is to field test the BKD ELISA and to develop the ELISA tests for the remaining pathogens to the point of field testing in the third year.

2.3 CONTROL OF IHN BY BROODSTOCK CULLING AND ANTIVIRAL DRUGS TO CONTROL IHN VIRUS IN SOCKEYE AND CHINOOK SALMON AND STEELHEAD TROUT (PROJECT NO. 82-21); Dan Mulcahy, U.S. Fish and Wildlife Service, National Fishery Research Center, Bldg. 204, Naval Station, Seattle, Washington 98115; (206) 527-6282 or (FTS) 446-6282; (Project Officer: Jerry Bouck)

Infectious hematopoietic necrosis (IHN) virus was responsible for the loss of more than 15 million salmonid fish in 1980 and 1981. In an effort to reduce the losses due to this disease, a technique called brood-stock culling was developed. In this procedure, fish are spawned individually, a sample taken for viral testing analysis, and the eggs are held in separate incubators. When the results of viral testing are known, the eggs from the carrier fish are destroyed and those from the negative fish are retained for culture. Losses of steelhead trout fry at Cowlitz Hatchery (Washington Department of Game) were 60 percent the year before culling began, but dropped to 10 percent and 0 percent after culling was done for the next 2 years. The procedure was tested on a large scale at Leavenworth National Fish Hatchery, where more than 4,500 samples were processed in 1 month. Although shown to be operationally and technically feasible on that scale, no viral challenge occurred to test the technique.

IHN virus adsorbs to the surface of salmonid fish sperm, and such adsorption may represent the mechanism for vertical transmission of the virus. Adsorption occurred extremely rapidly, with more than 99 percent of the virus attaching in less than 1 minute. The adsorption occurs with sperm from all salmonid species tested. Electron microscopy showed that the blunt end of the virus adsorbed to the head of the sperm. Testing of antiviral drugs will shift in emphasis and seek compounds to remove sperm with adsorbed virus from eggs during the water-hardening process.

Virus attached to sperm probably cannot be isolated by present procedures. Sampling spleens from male sockeye salmon gave an IHN virus incidence of 92 percent, kidneys, 67 percent, and sperm (homogenized), seminal plasma, or milt were 0 percent positive. Spleens from infected steelhead trout males were not always virus-positive, so both milt and spleen samples should be done where possible to test male fish.

Accurate IHN virus infection rates were determined for several populations of Columbia River salmonids. There was a range in infection rates from as low as 17 percent in Cowlitz summer steelhead trout in 1981 to 100 percent in Dworshak Hatchery steelhead trout in 1983. Of historical interest was the first examination of Columbia River sockeye salmon in recent years. The infection rate in White River sockeye was 61 percent in females and 65 percent in male fish. Variations in the proportion of spawning fish with high levels of virus were seen between populations examined, and may help explain variations in mortality rates.

2.4 EPIDEMIOLOGY AND CONTROL OF INFECTIOUS DISEASES OF SALMONIDS IN THE COLUMBIA RIVER BASIN (PROJECT NO. 83-312); John Rohovec and John Fryer, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331-3804; (503) 754-4441; (Project Officer: Jerry Bouck)

Ceratomyxa shasta: Rainbow trout highly susceptible to C. shasts were exposed at several locations in the Columbia River and also in the Snake, Imnaha, Grande Ronde, and Wallowa tributaries. The only fish found infected with this disease were from McNary Dam indicating an extension of the range of the infectious stage of this parasite. A high level of resistance to ceratomyxosis was determined in the following hatchery strains: Carson, Imnaha, Lookingglass, and Up-River Bright chinook and Sandy coho salmon; Inmaha and Wallowa steelhead trout.

Salmonid smolts were collected periodically from purse and beach seine catches in the Lower Columbia River between May and September 1983. The C. shasta infection frequency in 0-age chinook salmon ranged from 0 to 20 percent in purse seine samples and 1 to 24 percent in beach samples.

Reinbacterium salmoninarum: Kidney smears taken from salmonids captured in the open ocean were examined for R. salmoninarum. Positive kidney smears were found in chinook (14 percent), chum (3 percent), coho (3 percent), sockeye (4 percent), and salmon and steelhead trout (3 percent). Kidney smears were also taken from smolts seined from the Columbia River just prior to entering the estuary. After holding these fish for 180 days, mortalities were examined for R. salmoninarum. Positive kidney smears were found in the beach seined chinook salmon (10 percent), in purse seined chinook (18 percent), coho (8 percent), and salmon and steelhead trout (20 percent).

Examination of cryostat sections from samples of eggs from R. salmoninarum infected adults revealed the presence of R. salmoninarum on or in the egg wall.

Infectious Hematopoietic Necrosis Virus: Rainbow trout eggs were inoculated with $10^5$ IHNV particles and incubated in virus-free well water at the OSU-FDL. Results of daily sampling indicate virus titers decreased rapidly and virus was no longer detectable after 20 days of incubation.

Spawning steelhead trout from Round Butte were individually sampled for IHNV. Depending on the egg take, 54 to 60 percent of the females and 21 to 29 percent of the males were positive for virus. Eggs from these fish were divided into several subgroups. One was fertilized, water-hardened, and incubated using UV treated water. The other subgroup uses regular hatchery water. Data from these groups will give an indication of the effectiveness on UV sterilization in preventing IHNV epizootics.

In a second experiment, individual fish were examined for IHNV. To the extent possible, high titer IHNV and no or low titer mating pairs were selected. Eggs from these pairs will be hatched and fingerlings raised in UV treated water. Data from these groups will yield information valuable in determining whether or not propagation of eggs from IHNV negative adults will prevent IHNV epizootics.

## 3.0 FISH PHYSIOLOGY SECTION

3.1 COLUMBIA RIVER SALMONID OUTMIGRATION: McNARY DAM PASSAGE AND ENHANCED SMOLT QUALITY (PROJECT NO. 82-6); Carl B. Schreck and Hiram W. Li, Oregon Cooperative Fisheries Research Unit, 104 Nash Hall, Oregon State University, Corvallis, Oregon 97331-3803; (503) 754-4531; (Project Officer: Jerry Bouck)

The goal of this project is to increase the yield of Columbia River salmonids by facilitating smolt outmigration. Toward this goal our objectives were to evaluate the stress to smolts at the McNary Dam collection and transportation system, and to propose methodologies that minimize stresses within that system. We collected outmigrant, spring and fall chinook from various points in the collection, transportation system and to examined physiological indices of stress (plasma cortisol, hepatic glycogen, among others) and fish performance capacities in challenge tests (saltwater challenge, secondary stress, among others). Fish were sampled on the upstream side of the dam (gatewells), after they went through the bypass system (before the bar-sorter), just before they entered the raceway, before and after they were loaded on to the transport vehicle (truck or barge) and after transport to Bonneville Dam. Furthermore, fish taken from these sites were held in plastic tanks with flow-through water and serially sampled through 72 h to evaluate recovery rates. The results were then examined in terms of the relative magnitude and duration of the changes in the physiological indices and performance capacities.

We have reached the following conclusions based on our 1983 sampling:

1. After the smolts entered the raceway or were transported to Bonneville Dam, 12 to 24 h were required before the physiological indices returned to prestress levels.

2. Stress from marking takes at least 24 h for recovery of physiological indicators of stress.

3. The ability of fish to respond to the collection/transport stress, changes with ontogenetic development, and environmental conditions.

4. The stressful elements of the dam's collection system are additive both in terms of smolts' physiological response and their response to a secondary stress. Thus, reduction in the stressfulness of any part of the system will result in a reduction in the total stress of the system and should increase in the fishes' performance capacities.

5. The environment of the transport vehicles appears to mediate the stress response and elements of this environment (e.g., darkness) maybe adaptable to the collection facility.

3.2 EFFECTS OF STRESS ON THE VIABILITY OF CHINOOK SALMON AND SMOLTS TRANSPORTED FROM THE SNAKE RIVER TO THE COLUMBIA RIVER ESTUARY (PROJECT NO. 82-5); Jim Congleton, U.S. Fish and Wildlife Service/Cooperative Fishery Research Unit; University of Idaho, Moscow, Idaho 83843; (Project Officer: Jerry Bouck)

Transportation of chinook salmon smolts from Snake River dams to the Columbia estuary has not reversed the downward trend of Idaho chinook salmon stocks. For this reason, we investigated some possible effects of transportation-related stress on viability of chinook salmon. Two subprojects dealt with the effects of stress on the ability of juvenile chinook salmon to avoid capture by predatory fish and to resist bacterial kidney disease after acclimation to sea water. A third subproject attempted to confirm the linkage between exposure to stress and decreased smolt-to-adult survival. The fourth subproject examined effects of ambient salinity on stress response in transported chinook salmon.

Vulnerability to predation increased in all stocks of spring chinook salmom tested following crowding at high densities and long durations of crowding. Predation rates and plasma cortisol concentrations were significantly correlated in trials with the two most extensively tested stocks of chinook salmon. Chinook salmon with plasma cortisol concentrations of 75-150 ng/ml and above were captured by predators at a higher rate than unstressed control fish. Plasma cortisol concentrations in transported chinook salmon frequently exceed 150 ng/ml at the time of release. Stress associated with transportation probably impairs the ability of transported fish to escape attacks of predators immediately after release, but losses would depend upon numbers of predators near release sites.

Effects of stress from transport of chinook salmon smolts on subsequent rearing and survival in seawater were evaluated by transporting fish from hatcheries to a seawater lab for 78-131 days of rearing. High quality smolts survived the stresses of transportation and conversion to seawater at a high rate. Lesser quality smolts, as measured by performance in the hatchery, survived at lower, probably unacceptable, rates during seawater rearing.

The planned release of marked stressed and unstressed chinook smolts from Eagle Creek National Fish Hatchery was not achieved in 1983. A release of marked stressed and unstressed fish will be made in the spring of 1984. Comparison of adult return rates from the two qroups will indicate whether or not prerelease exposure to stress adversely affects survival.

Following transportation and handling, plasma cortisol declined more rapidly in chinook salmon smolts held in 5 or 10 ppt seawater than in smolts fresh water or 20 ppt seawater. Recovery of depressed plasma $Na^+$ concentrations following transportation was slowest in fish held in fresh water and fastest in fish held in 20 ppt seawater. Use of 5 ppt seawater (or salt water) during transport of fish by truck would minimize mortality due to stress-caused ionoregulatory disturbance. Release of barged chinook smolts into brackish water would speed recovery of stress hormone and blood electrolyte concentrations to "unstressed" levels.

3.3 DEVELOPMENT OF TRANSPORT MEDIA TO REDUCE STRESS AND IMPROVE SMOLT SURVIVAL IN HAULING JUVENILE CHINOOK SALMON (PROJECT NO. 82-4); Gary A. Wedemeyer, U.S. Fish and Wildlife Service, Bldg. 204, Naval Station; Seattle, Washington 98115; (205) 527-6282; (Project Officer: Jay Dugoni)

Selected combinations of mineral salts ($Na^+$, $Cl^-$, $Ca^{++}$. $PO_4^{-}$, $HCO_3^-$, and $Mg^{++}$) plus tranquilizing concentrations of MS-222 were tested for ability to mitigate stress and improve smolt survival during hauling of spring chinook salmon (Oncorhynchus kisutch). Effectiveness in reducing acute stress (handling, crowding) was evaluated by measuring the severity of the generalized stress response and of the accompanying osmoregulatory disturbances. Blood glucose, chloride, cortisol, liver glycogen, and muscle ATP were monitored as well as survival itself. Effects on ability to survive and grow for 4 months in full strength (28°/00) sea water were used as the final criteria of success.

Of the 14 formulations tested, MS-222 at 10 mg/L and a combination of 10 mg/L MS-222 plus 250 mg/L $NaHCO_3$ emerged as top-rated in terms of ability to mitigate against acute stress during transport at a density of 0.5 lbs/gal. Benefits to 4-mo seawater growth and survival after hauling were minimal. Completion of the second (and final) year of the project will be required in order to provide firm guidelines on alternatives, benefits, and consequences.

3.4 BIOENERGETICS OF JUVENILE SALMON DURING THE SPRING OUTMIGRATION (PROJECT NO. 82-11); Dennis W. Rondorf, Project Leader, National Fisheries Research Center, USFWS - Willard Substation; Star Route, Cook, Washington 98605; (FTS) 422-7956 or (509) 538-2299; (Project Officer: Jerry Bouck)

This 3-year study was initiated to determine if impoundments in the Columbia Basin have increased the energy requirements, and consequently decreased survival, of juvenile spring chinook salmon (Oncorhynchus tshawytcscha) during seaward migration. We hypothesize that additional energy may be required by upper river smolts because: (a) outmigration time has been increased as much as 100 percent by impoundments, particularly during low-flow years; (b) water temperatures are slight increased related to the above-noted delay and seasonal warming; (c) additional energy is expended on active swimming in reservoirs; and (d) food consumption is low during the spring outmigration, especially for hatchery-produced smolts. This study will formulate a bioenergetics model to simulate the energy budgets of juvenile chinook salmon as a function of migration rate, temperature, and flow regimes.

A physiological approach to bioenergetics of juvenile salmon was chosen and the energy budget was divided into the following components: $C = B + R + U + F$ where: C = food consumption; B = energy in biomass (growth); R = respiration; U = excretory products; F = Faecal waste. Food consumption by juvenile spring chinook salmon during the seaward migration was estimated to be 4.2 percent of body weight per day. Daily food consumption was estimated at 873 cal/day/fish. Classification of the fish by origin, hatchery and wild, using a discriminant function developed from scale characteristics indicated wild fish consumed significantly more food than hatchery fish despite their smaller size.

Estimates of energy in fish biomass (B) during the outmigration will be used to validate energetics of fish simulated by the model. Energy density (kcal/g) of juvenile spring chinook salmon decreased about 18 percent during migration due to a precipitous decline in lipids. Whole body concentration of lipid in juvenile spring chinook salmon declined about 55 percent after juveniles migrated approximately 50 percent of the distance to the estuary. Increased fish weight, however, precluded a decline in total caloric value. The decline in lipids and energy density and the associated increase in fish weight was partially attributed to the parr-smolt transformation during the first half of the migration. Future project activities will develop model parameters for the respiration component (R) and migration scenarios for final simulations.

3.5 INVESTIGATION OF THE PROCESS FOR REGISTRATION OF SQUOXIN FOR CONTROL OF SQUAWFISH (PROJECT NO. 83-428); Robert Rulifson, 14232 SE. 23, Bellevue, Washington 98007; (206) 746-5192; (Project Officer: Dale Johnson)

DESCRIPTION OF PROJECT

The objectives of this project are to assemble all available information on squawfish and squoxin and prepare a bibliographic index and evaluate regulatory considerations for registration with EPA and recommend an approach.

ACCOMPLISHMENTS

1. Information has been assembled on squawfish and squoxin from published and unpublished literature using public, agency, and private libraries. Searches of 11 data banks have been conducted. Copies of pertinent literature have been procured and a bibliography of 229 references has been compiled with abstracts for preparation of a bibliographic index.

2. Literature pertaining to chemical structure and toxicity/ bioaccumulation has been reviewed and discussed with several fish toxicologists.

3. The oxidative byproducts of squoxin in water have been identified as 1, 2 naphoquinone and 1 hydroxymethyl-2 naphthol (not known carcinogens).

4. Rules and regulations for registration of pesticides with EPA have been assembled and analyzed. Specific requirements for registration of squoxin have been tentatively identified. A summary of information was prepared for each data requirement of EPA and discussed with them on February 15, 1984.

5. Meetings have been held with many individuals and groups having expertise pertinent to squoxin registration. These include NMFS, OSU, FWS (LaCrosse), IR-4 (Rutgers University), EPA (Reg. X), Crain MacPhee, Leon Terriere, and others.

6. Good Laboratory Practice Rules have been reviewed. The final rules are more reasonable than those proposed in 1979.

7. A state pesticide-use permit was investigated as an alternative. This is only possible with prior registration by EPA except for emergency use.

PRELIMINARY CONCLUSIONS

1. Much of the initial petition to EPA by IR-4 in 1977 was inadequate or incomplete to meet EPA requirements for registration. Some of the incomplete data is available and can be submitted to upgrade the previous submittal.

2. Considerable additional research will be required in the areas of product chemistry, fish metabolism, and environmental fate.

3. The original manufacturer (American Cyanamid) cannot be counted on for support because there is no profit motive. Squoxin can be easily manufactured in a one-step process.

4. An experimental-use permit from EPA will be required to conduct further field research.

5. The EPA review process is fragmented; up to 12 specialists comment on material in their specialty. EPA will not identify the remaining specific research needed to meet their requirements, but will identify the subject areas. Setting up a testing program and submittal of results would require substantial coordination with EPA review staff.

6. Complying with EPA requirements will satisfy FDA requirements.

FORTHCOMING ACTIVITIES

1. Complete bibliographic index.

2. Define the pesticide registration process; identify data requirements lacking for registration; estimate cost for conducting necessary studies; and estimate cost of application of squoxin.

3. Determine the major biological and chemical issues with squoxin.

4. Identify other statutory and social requirements and conflicts and recommend solutions. Identify additional environmental requirements of state and Federal governments and define steps to satisfy.

5. Prepare final report.

3.6 IMPRINTING OF HATCHERY-REARED SALMON AND STEELHEAD FOR HOMING OF TRANSPORTED FISH (PROJECT NO. 78-1); Emil Slatick, National Marine Fisheries Service, P.O. Box 267, Clarkston, Washington 99403; (509) 758-3349; (Project Officer: Tom Vogel)

The ability to activate the imprint mechanism at the proper time should assure a suitable homing cue which coupled with transportation may result in high smolt survival and better returns to the homing site or hatchery. In our study four major imprint techniques were used to cue juveniles: natural migration, short distance migration, single exposure, and sequential exposure to unique water supplies. During the 3-year marking phase of the program, a total of over 4 million juvenile salmon and steelhead were marked and released in 23 experiments.

The experimental treatments provided varying degrees of success in both homing and survival of adults. Examples include: (a) fish which did not receive a homing imprint before being transported returned to and remained in the vicinity of their release site; (b) some fish appeared to receive a partial imprint--these fish migrated over 300 miles up the Columbia River but did not return to their homing site; and (c) portions of some groups of fish recieved a positive homing cue--many of these fish migrated up to 500 miles in returning back to their hatchery homing site.

For this presentation I will review the techniques we used and summarize the findings for a stock of coho salmon and fall chinook slamon released in 1980.

GBouck:ay (WP-PJS-3129N)